FORM PTO-1390 (REV 11-98)

U S DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: **REISNER=5**

U.S. APPLICATION NO (If known, see 37 CFR 1.5): **09/202181**

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/IL97/00184	**10 June 1997**	**11 June 1996**

TITLE OF INVENTION
Yair REISNER et al.

APPLICANT(S) FOR DO/EO/US
HUMAN MONOCLONAL ANTIBODIES TO THE HEPATITIS B SURFACE ANTIGEN

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☒ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☒ A FIRST preliminary amendment.
    ☐ A SECOND or SUBSEQUENT preliminary amendment.
14. ☐ A substitute specification.
15. ☐ A change of power of attorney and/or address letter.
16. ☒ Other items or information:

1. **A courtesy copy of the specification as originally filed.**
2. **Small entity statements - small business concern.**
3. **A courtesy copy of the first page of the International Publication (WO97/47654).**
4. **A courtesy copy of the International Search Report.**
5. **A courtesy copy of the International Preliminary Examination Report with annexes.**
   Note: Please use the claims as they appear in the IPER annexes as the claims this case.
6. **Formal drawings, 10 sheets, figures 1-11.**

U.S. APPLICATION NO (if known, see 37 CFR 1 5)	INTERNATIONAL APPLICATION NO PCT/IL97/00184	ATTORNEY'S DOCKET NUMBER REISNER=5

17. ☒ The following fees are submitted:	CALCULATIONS PTO USE ONLY	
BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :		
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO **$970.00**		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO **$840.00**		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO **$760.00**		
International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) **$670.00**		
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4) **$96.00**		
ENTER APPROPRIATE BASIC FEE AMOUNT =	**$ 840.00**	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	**$**	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	**18** - 20 =		X **$18.00**	**$**	
Independent claims	**5** - 3 =	**2**	X **$78.00**	**$ 156.00**	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ **$260.00**	**$**	
TOTAL OF ABOVE CALCULATIONS =				**$ 996.00**	
Reduction of 1/2 for filing by small entity, if applicable. A Small Entity Statement must also by filed (Note 37 CFR 1.9, 1.27, 1.28).				**$ - 498.00**	
SUBTOTAL =				**$ 498.00**	
Processing fee of **$130.00** for furnishing the English translation later than ☐20 ☐30 months from the earliest claimed priority date (37 CFR 1.492(f)). +				**$**	
TOTAL NATIONAL FEE =				**$ 498.00**	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property +				**$**	
TOTAL FEES ENCLOSED =				**$ 498.00**	
				Amount to be: refunded	**$**
				charged	**$**

a. ☒ A check in the amount of $ **498.00** to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. _____ in the amount of $_____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. **02-4035**. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

BROWDY AND NEIMARK, P.L.L.C.
419 Seventh Street N.W., Suite 300
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SIGNATURE:

Roger L. Browdy
NAME

25,618
REGISTRATION NUMBER

Date of this submission: December 10, 1998

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	)	Art Unit:
Yair REISNER et al.	)	
IA No.: PCT/IL97/00184	)	Washington, D.C.
IA Filed: 10 June 1997	)	
U.S. App. No.: (Not Yet Assigned)	)	December 10, 1998
National Filing Date: (Not Yet Received)	)	
For: HUMAN MONOCLONAL...	)	Docket No.: REISNER=5

PRELIMINARY AMENDMENT

Honorable Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

Contemporaneous with the filing of this case and prior to calculation of the filing fee, kindly amend as follows:

IN THE CLAIMS

Claim 3, line 1, delete "or 2".

Claim 4, line 1, delete "Claims 1-3 ", and insert therefor --claim 1--.

Claim 9, line 3, delete "and/or 6".

Claim 10, line 3, delete "and/or 6".

Claim 11, line 2, delete "and/or 6".

Claim 12, line 3, delete "or 6".

Claim 13, line 1, delete "or 6".

Claim 15, line 3, delete "or 6".

Claim 17, line 3, delete "any one of claims 9, 15 or 16", and insert therefor --claim 9--.

Claim 18, line 2, delete "or 6".

REMARKS

The above amendments to the claims are being made in order to eliminate any properly multiply dependent claims, for the purpose of reducing the filing fee. Please enter this amendment prior to calculation of the filing fee in this case.

Favorable consideration and allowance are earnestly solicited.

Respectfully submitted,

BROWDY AND NEIMARK, P.L.L.C.
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ANTI HBV ANTIBODIES

FIELD OF THE INVENTION

The present invention concerns a process for obtaining hybridoma cell lines which produce human antibodies capable of binding to the hepatitis B virus surface antigen, the hybridoma cell lines, antibodies produced by the cell lines, and various uses thereof.

BACKGROUND OF THE INVENTION

Hepatitis B virus (HBV) infection is a major worldwide health problem. Approximately 5% of the world population is infected by HBV and chronically infected patients carry a high risk of developing cirrhosis and hepatocellular carcinoma. (Progress in Hepatitis Research: Hepatitis B virus (HBV), Hepatitis C virus (HCV) and Hepatitis Delta virus (HDV) Ed. O. Crivelli, Sorin Biomedica, 1991).

The immune response to HBV-encoded antigens includes both a cellular immune response which is active in the elimination of HBV infected cells, as well as a humoral antibody response to viral envelope antigens which contributes to the clearance of circulating virus particles. The dominant cause of viral persistence during HBV infection is the development of a weak antiviral immune response.

Recombinant HBV vaccines provide a safe and effective means for active immunization against HBV, however, they do not always induce a sufficient and rapid antibody response.

Interferon-α has been used in the therapy of Hepatitis B infection showing an efficacy of only 30-40% in highly selected patients.

In addition, passive immunization with human polyclonal anti Hepatitis B antisera has been shown to be effective in delaying and even preventing recurrent HBV infection (Wright, T.L. and Lau, J.Y.N. The Lancet **342**:1340-1344,

(1993)). Such human polyclonal antisera are prepared from pooled plasma of immunized donors. These preparations are very expensive and available in relatively small amounts. Furthermore, pooled plasma may contain contaminated blood samples and thus treatment with such antisera increases the patient's risk to contract other viral infections such as hepatitis C or HIV.

An alternative approach for the treatment of HBV infection is the use of monoclonal antibodies (MoAb).

PCT patent application PCT/NL94/00102 discloses human monoclonal antibodies directed against Hepatitis B surface antigen which are secreted by the hybridoma cell lines Mab 4-7B and Mab 9H9. The monoclonal antibody secreted by the cell line Mab 4-7B recognizes a linear epitope of HBVsAg and is different from the Mab 9H9 monoclonal antibody which recognizes a conformational epitope. The antibodies are claimed for simultaneous use in the treatment of chronic Hepatitis B infections.

PCT patent application PCT/US92/09749 discloses human monoclonal antibodies against HBVsAg which are secreted by the hybridoma cell lines PE1-1, ZM1-1, ZM1-2, MD3-4 and LO3-3. The antibodies bind to different HBV epitopes and are used for reducing the level of circulating HBVsAg.

Japanese Patent Application JP 93066104 discloses a hybridoma of a human lymphocyte cell strain TAW-925 and a human lymphocyte transformed by Epstein-Barr virus. The hybridoma produces a human monoclonal antibody against HBVsAg.

U.S. Patent Application No. 4,883,752 discloses preparation of human-derived monoclonal antibody to HBVsAg, by administration of HBVsAg vaccine to humans, recovering their lymphocytes, stimulating the lymphocytes *in vitro* by a non specific stimulator, fusing said cells with a myeloma cell, and selecting for hybridomas with secrete anti HBVsAg antibodies.

Ichimori *et al., Biochem. and Biophysic. Research Communications* **129**(1):26-33, 1985 discloses a hybridoma secreting human anti HBVsAg monoclonal antibodies which recognize the a-determinant of HBVsAg. Later, Ichimori, *et al., supra* **142**(3):805-812, 1987 disclosed another hybridoma which stably secretes human monoclonal antibody against HbsAg.

The abovementioned antibodies were all developed by *in vitro* immortalization of antibody-producing cells from individuals positive for anti-HBV antibodies.

A new approach enabling adaptive transfer of human peripheral blood mononuclear cells (PBMC) into lethally irradiated normal strains of mice radioprotected with severe combined immune deficiency (SCID) bone marrow was recently described (Lubin I., *et al., Blood*, **83**:2368, 1994). Secondary humoral responses to various recall antigens as well as a primary humoral response to other antigens were shown to be generated effectively in such human/mouse chimeras (Marcus H., *et al., Blood*, **86**:398-406, 1995).

SUMMARY OF THE INVENTION

In accordance with the present invention, it was found that hybridoma cell lines secreting human antibodies capable of binding to the Hepatitis B surface antigen (HBVsAg) may be obtained using the above mentioned human/mouse chimeras. In accordance with the present invention, human peripheral blood lymphocytes (PBL) from human donors positive for anti HBVsAg antibodies are engrafted into normal strains of mice which were lethally irradiated and radioprotected with SCID bone marrow. After immunization of such chimeric mice with HBVsAg, human cells are obtained from the mice spleens and fused *in vitro* with heteromyeloma cells to generate hybridomas secreting human antibodies having a high affinity and specificity to HBVsAg.

The present invention thus provides a process for obtaining human monoclonal antibodies (hMoAb) capable of binding to Hepatitis B virus surface antigen (HBVsAg) comprising:

(a) immunizing a chimeric rodent M4 having xenogeneic hematopoietic cells with Hepatitis B surface antigen (HBVsAg) such that xenogeneic antibody-producing cells are produced in said rodent, wherein said rodent M4 is a rodent M1, the hematopoietic cells of which have been substantially destroyed, said rodent M1 having transplanted therein hematopoietic cells derived from a mouse M2 having a hematopoietic deficiency, and xenogeneic hematopoietic cells derived from human M3;

(b) removing and immortalizing said antibody-producing cells;

(c) selecting and cloning the immortalized antibody producing cells producing the antibodies capable of binding to HBVsAg and;

(d) isolating the antibodies produced by the selected, cloned immortalized antibody producing cells.

In accordance with the invention, spleens of the immunized chimeric rodent M4 are removed between 12 and 20 days after human PBL transplantation, preferably at day 14 after transplantation thereof. Cell suspensions are prepared from the spleens and the antibody producing cells obtained from the immunized chimeric rodent M4 are fused preferably with a human-mouse fusion partner such as a heteromyeloma by techniques well known in the art (e.g. Köhler & Milstein, *Nature*, **256**:495-497, 1975). In order to isolate the antibodies produced by the selected hybridoma cell lines in accordance with the invention, the hybridoma cell lines are either cultured *in vitro* in a suitable medium wherein the desired monoclonal antibody is recovered from the supernatant or, alternatively, the hybridoma cell lines may be injected intraperitoneally into mice and the antibodies harvested from the malignant ascitis or serum of these mice. The supernatant of the hybridoma cell lines are first screened for production of human IgG antibodies by any of the methods known in the art such as enzyme linked immunosorbent assay (ELISA) or radioimmuno assay (RIA). Hybridomas testing positive for human IgG are then further screened for production of anti HBVsAg antibodies by their capability to bind to HBVsAg.

The M1 rodent in accordance with the invention is preferably a rodent conventionally used as a laboratory animal, most preferably a rat or a mouse.

The mouse M2 may have any hematopoietic deficiency including genetic hematopoietic deficiencies as well as induced hematopoietic deficiencies. Non limiting examples of hematopoietic deficiencies include SCID, Bg, Nu, Xid or mice having any combination of the abovementioned hematopoietic deficiencies. In addition, the hematopoietic deficiency may also be a result of gene deletion or transgenic mice may be used.

The hematopoietic cells derived from the donor mouse M2 are preferably bone marrow cells either untreated or depleted of T cells. Other suitable sources of hematopoietic cells which may also be used include, for example, spleen cells, fetal liver cells or peripheral blood cells.

The xenogeneic hematopoietic cells derived from the human M3 are preferably PBL cells but may also be derived from any suitable source of human hematopoietic cells such as bone marrow cells, cord blood cells, thymus spleen or lymphnode cells, etc.

By a most preferred embodiment, the rodent M1 is a mouse or rat, the mouse M2 is a SCID mouse and the xenogeneic hematopoietic cells derived from the human M3 are PBLs from a human M3 which has already been exposed to the

HBVsAg either spontaneously as a result of a prior infection or induced following vaccination. Such humans will have a relatively high titer of anti HBVsAg antibodies as compared to individuals which have never been infected with HBV and, therefore, when PBLs from such donors are used as M3 donor cells in accordance with the present invention, the immunization of the M4 chimeric mouse with HBVsAg will elicit a secondary immune response of the transplanted human PBLs in the M4 chimeric mouse. A most preferred human donor M3 is such which tested negative for the HB virus but shows a high titer of antibodies against HBVsAg. Such PBLs from the human M3 donor may be obtained either by whole blood donation or by leukophoresis.

The HBVsAg used for immunizing the chimeric rodent M4 in accordance with the invention is preferably a Hepatitis B virus vaccine containing the purified major surface antigen of the virus prepared by recombinant DNA technology (Engerix™-B, SIB Biological (Rixensart, Belgium)).

The present invention is also directed to hybridoma cell lines producing human monoclonal antibodies capable of binding to HBVsAg, as well as to human monoclonal antibodies capable of binding to HBVsAg and fragments thereof substantially maintaining the antigen binding characteristics of the whole antibody. Such fragments may be, for example, Fab or $F(ab)_2$ fragments obtained by digestion of the whole antibody with various enzymes as known and described extensively in the art. The antigenic characteristics of an antibody are determined by testing the binding of an antibody to a certain antigenic determinant using standard assays such as RIA, ELISA or FACS analysis.

Typically, the human monoclonal antibodies obtained by the method of the present invention have a relatively high affinity to HBVsAg being in the range of about 10^{-9}M to about 10^{-10}M as determined in a competative ELISA assay.

In accordance with a specific embodiment of the present invention there are provided hybridoma cell lines designated herein as *"18.5.1013"* and *"19.79.5"* which were deposited on May 22, 1996, in the European Collection of Cell Cultures (ECACC, CAMR, Salisbury, Wiltshire, SP40JG, U.K.) under Accession Nos. 96052170 and 96052168, respectively. Anti HBVsAg human monoclonal antibodies secreted by the above hybridoma cell lines and designated herein as *"Ab18.5.1013"* and *"Ab19.79.5"*, respectively, are also provided as well as fragments thereof retaining the antigen binding characteristics of the antibodies, and antibodies capable of binding to the antigenic epitope bound by *"Ab18.5.1013"* and

"Ab19.79.5".

The antigen bound by the antibodies defined above also constitutes an aspect of the invention.

Further aspects of the present invention are various diagnostic, prophylactic and therapeutic uses of the human anti HBVsAg monoclonal antibodies and the Ag bound by them. In accordance with this aspect of the invention, pharmaceutical compositions comprising the human anti HBVsAg monoclonal antibodies may be used for the treatment of chronic Hepatitis B patients by administering to such a patient a therapeutically effective amount of the monoclonal antibody or portion thereof capable of binding to the HBVsAg being an amount effective in alleviating the symptoms of the HBV infection or reducing the number of circulating viral particles in an individual.

Such pharmaceutical compositions may comprise one or more antibodies of the invention. In addition to the antibodies of the invention the pharmaceutical compositions may optionally also comprise a carrier selected from any of the carriers known in the art. One example of such a carrier is a liposome. The pharmaceutical compositions of the invention may also comprise various diluents and adjuvants known per se.

The compositions of the invention may be administered by a variety of administration modes including parenterally, orally etc.

Compositions comprising the antibodies of the invention, as described above, may be administered in combination with other anti viral agents. Such agents may include, as a non limiting example: Interferons, anti HB monoclonal antibodies, anti HB polyclonal antibodies, nucleoside analogs, and inhibitors of DNA polymerase. In the case of such a combination therapy the antibodies may be given simultaneously with the anti viral agent or sequentially either before or after treatment with the anti viral agent.

The pharmaceutical compositions of the invention may also be used, for example, for immunization of new born babies against HBV infections or for immunization of liver transplantation patients to eliminate possible recurrent HBV infections in such patients.

By a further embodiment, the antibodies of the invention may also be used in a method for the diagnosis of HBV infections in an individual by obtaining a body fluid sample from the tested individual which may be a blood sample, a lymph sample or any other body fluid sample and contacting the body fluid sample with a human anti HBVsAG antibody of the invention under conditions enabling the

formation of antibody-antigen complexes. The level of such complexes is then determined by methods known in the art, a level significantly higher than that formed in a control sample indicating an HV infection in the tested individual. In the same manner, the specific antigen bound by the antibodies of the invention may also be used for diagnosis. In the same manner, the specific antigen of the invention may also be used for diagnosis of HBV infection in an individual by contacting a body fluid sample with the Ag and determining the presence of Ag-Ab complexes in the sample as described above. In addition, the Ag of the invention may be used for immunizing an individual to elicit a humoral response against HBV.

The present invention further provides a kit for use in the therapy of HBV infections or diagnosis of such infections comprising the antibodies of the invention, the antigen bound by the antibodies of the invention and any further reagents necessary for detecting such antibodies or antigens in a tested sample.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a graphic representation showing the amount of total human Ig (mg/ml) and the amount of specific anti HBs antibodies (mU/ml) in the sera of irradiated mice which were radioprotected with SCID bone marrow (chimeric mice).
PBL+Engerix: the chimeric mice were further transplanted with human PBL from donors positive for anti HBs antibodies, and vaccinated with Engerix B in an aluminum hydroxide adjuvant (alum).
PBL+Alum: the chimeric mice were further transplanted with human PBL from donors positive for anti HBs antibodies, and vaccinated with Alum alone (no Engerix B).
SCID-BM+Engerix: the chimeric mice were vaccinated with Engerix B (no transplantation of human PBL).
SCID-BM+Alum: the chimeric mice were vaccinated with Alum (no human PBL and no Engerix B).
The black line represents the initial level of anti HBs antibodies in the serum of the human PBL donor.

Fig. 2 is a graphic representation showing the specific activity, i.e. the levels of anti HBVs antibodies per mg of human Ig in the sera of human donors (A-D, black columns) and the specific activity in the sera of chimeric mice transplanted respectively with human PBL of said donors (A-D, striped columns).

Fig. 3 is a graphic representation showing time response curve of anti HBs antibodies specific activity (mU/mg) in sera of chimeric mice (dotted line). The

black columns represent the level of total human Ig (mg/ml), and the striped columns represent the level of specific anti HBs antibodies (mU/ml).

Fig. 4 is a graphic representation showing competitive inhibition of binding of anti HBs antibodies to HBs particles. The extent of binding was measured by ELISA using a horseradish peroxidase labeled anti human IgG secondary antibody. The anti HBs antibodies were diluted as indicated in the graph in medium (empty squares) or in 0.5 μg/ml HBs particles (black squares).

Fig. 5 is a photograph showing Hepatitis B infected liver sections stained with anti HBVs antibodies. All sections were stained with a *"secondary"* antibody, i.e. goat anti human Ig conjugated to biotin.

A - negative control. No first antibody.

B - positive control. First antibody - mouse anti HB antibody and a secondary anti-mouse Ig.

C - staining with anti HBs antibody No. 19.79.5.

D - staining with anti HBs antibody No. 18.5.1013.

Reference will now be made to the following Examples which are provided by way of illustration and are not intended to be limiting to the present invention.

Fig. 6 is a schematic representation of the binding of Ab 19.79.5 to a set of 15 well characterized HBsAg types. The y axis represents optical density units. The x axis represents different HBsAg types.

Fig. 7 is a graphic representation of the percentage of HBV infected animals at days 11 and 18 in the untreated group and Ab 18.5.1013 treated group (in the inhibition model).

Fig. 8 is a graphic representation of the percentage of HBV infected animals at days 10 and 17 in the untreated group and Ab 19.79.5 treated group (in the combined prophylaxis/inhibition model).

Fig. 9 is a graphic representation of the percentage of HBV infected animals at days 11 and 19 in the untreated group and Ab 19.79.5 treated group (in the combined inhibition/treatment model).

Fig. 10 Nucleic acid sequence and corresponding amino acid sequence of the light chain of the variable domain of Ab 19.79.5.

Fig. 11 Nucleic acid sequence and corresponding amino acid sequence of the heavy chain of the variable domain of Ab 19.79.5.

EXAMPLES

MATERIALS AND METHODS

Mice:

Animals used were 6-10 weeks old. BALB/c mice were obtained from Harlan (Weizmann Institute Animal Breeding Center (Rehovot, Israel)), SCID/NOD mice from the Weizmann Institute Animal Breeding Center (Rehovot, Israel). All mice were fed sterile food and acid water containing cyprofloxacin (20 µg/ml) (Bayer, Leverkusen, Germany). Whenever necessary, mice were injected daily with 1 mg Fortum i.p. for five days post BMT (Glaxo Operations UK, Greenford, England).

Conditioning Regimens:

BALB/c mice were exposed to total body irradiation (TBI), from a gamma beam 150-A 60Co source (produced by the Atomic Energy of Canada, Kanata, Ontario) with F.S.D of 75 cm and a dose rate of 0.7 Gy/min, with 4 Gy followed 3 days later by 10-11 Gy (split dose).

Preparation and Transplantation of Bone Marrow Cells:

The femoral and tibial bones were removed from mice and homogenized in a sterilized 50 ml Omni-Mixer stainless steel chamber (Omni-Mixer Homogeni æ, M del N . 17106, OMNI Int ernat ional, W t erbur y, CT. USA).

Recipient mice were injected i.v. with 4-6 x 10^6 of SCID/NOD bone marrow cells (in 0.2 ml PBS) immediately after irradiation.

Transplantation of Peripheral Blood Lymphocytes:

Peripheral blood lymphocytes (PBL) were obtained after informed consent by leukophoresis from donors positive for HBs antibodies and negative for HBV. PBLs were washed twice, counted and resuspended in PBS to the desired cell concentration.

100 x 10^6 human PBL were injected intraperitoneally (i.p.) into recipient mice, conditioned as described above. Control mice did not receive human PBL.

Immunization of the Chimeric Animals:

Mice were immunized once with hepatitis B vaccine (Engerix™-B; SB Biologicals Rixensart, Belgium) administered i.p. together with the PBL.

Cell and Plasma Collection from Human Mouse Chimera:

Animals were bled from the retro-orbital vein using heparin-coated glass capillaries. Plasma was kept for human-Ig determination. Spleens were removed after the animals were sacrificed by cervical dislocation, cut into pieces and pressed through stainless steel sieves to make a cell suspension in PBS.

Cell Fusion:

Cells were mixed with the human-mouse heteromyeloma HMMA2.11TG/0 (Posner *et al. Hybridoma*, 6:611-625, 1987) at 3:1 ratio. Fusion was performed with 50% (w/v) PEG 1500 (Boehringer Manheim GmbH) in a standard procedure. Fused cells were seeded at a concentration of 30000 cells/well in 96-well U-bottom microtiter plates (Nunc, Denmark) in complete medium containing HAT-supplement (1x) (Biological Industries, Beit Haemek, Israel). Cells were fed with fresh HAT-medium a week latter. Two weeks after fusion supernatants were harvested for ELISA and medium was replaced with fresh HT-medium.

Hybridoma cultures secreting specific anti-HBs Ig were cloned at 0.5 cell/well in 96-well U-bottom microtiter plates.

Determination of Human Immunoglobulin:

Sera were tested for antigen specific and total human Ig. Total human Ig was quantified by sandwich ELISA using goat F(ab)2-purified anti-human IgG+IgM+IgA (Zymed Laboratories, San Francisco, CA) as the capture agent and peroxidase-conjugated purified goat anti-human (Zymed Laboratories) as the detection reagent. Human serum of known immunoglobulin concentration was used as the standard (Sigma, Rehovot,Israel). Microplates (Nunc, Roskilde, Denmark) pre-coated with the capture reagent (2.5 ug/ml, 50 ul/well) and blocked with 1% BSA were incubated overnight at 4C with dilutions of plasma from 1:20000 to 1:640000, or the standard from 0.2 to 0.06 ug/ml, then washed 5 times with PBS-Tween solution. The detection reagent was added and the plates were incubated for 1h at 37C, then washed again 3 times. Fresh substrate solution (TMB, Sigma) was added and, after peroxidase-catalyzed color development, the reaction was stopped by addition of 10% sulfuric acid. Absorbance at 450 nm was quantified on an ELISA reader (Dynatech, Port Guernsey, Channel Islands, UK).

Concentration of antigen-specific human antibodies in mice sera was determined by HBsAb EIA kit (ZER, Jerusalem, Israel).

Human antibodies in hybridoma supernatants were determined by overnight incubation of supernatants on goat anti-human IgG+A+M (Zymed) coated plates, with goat anti-human IgG-peroxidase conjugated as the secondary reagent.

Antigen-specific antibodies in hybridoma supernatants were determined as above using Hbs antigen coated plates.

Determination of Human IgG Subclasses:

Human IgG subclasses were determined by sandwich ELISA using goat F(ab)2-purified anti-human IgG+IgM+IgA (Zymed Laboratories, San Francisco, CA) coated plates and Hbs antigen coated plates. Mouse anti-human IgG subclasses (Sigma) were used as second antibody and peroxidase-conjugated purified goat anti-human (Zymed Laboratories) as the detection reagent.

Statistic Analysis:

Statistical analysis was performed using the Stat View II program (Abacus Concepts, Inc., Berkeley, CA) on a Mackintosh Quadra 605 or Microsoft Excel 5.0 (Microsoft) on a 486 DX2 PC compatible. Student t-test, Anova correlation and regression analysis were utilized to calculate probability (p) and correlation coefficient (r) values. Results are presented as mean ± standard error.

Affinity Constant Measurements:

Determination of affinity constants (K_D) of the different anti-HBs antibodies to ad antigen (Chemicon Cat. No. AG 850) in solution were performed according to Friguet *et al.* (*Journal of Immunological Methods*, 77:305-319, 1985). The antigen at various concentrations ($3.5x10^{-10}$M to $1.4x10^{-9}$M) was first incubated in solution with a constant amount of antibody ($3.4x10^{-11}$M), in 0.1 M sodium phosphate buffer containing 2 mM EDTA and 10 mg/ml BSA, pH 7.8 (medium buffer). After o.n. incubation at 20 C the concentration of free antibody was determined by an indirect ELISA. A volume of 300ul of each mixture were transferred and incubated for 2h at 20 C into the wells of a microtitration plate (Nunc) previously coated with Ad (50 µl/well at 1 µg/ml in 0.1 M $NaHCO_3$ buffer, pH 9.6 for 2 h at 37°C). After washing with PBS containing 0.04% Tween 20, the bound antibodies were detected by adding HRP-$F(ab')_2$ Goat anti human IgG (Zymed) diluted 1:3000 with medium buffer, 50 µl/well 2 h at 20°C. The plate was developed with TMB chromogen (Sigma T-3405 tablets) 50 µl/well, the reaction stopped with 10% H_2SO_4 50 µl/well and the plate read in an ELISA reader at 450 nm. The conditions were chosen so that the resulting f values (see Friguet *et al.*) were around 0.1. The antibody concentration used was deduced from an ELISA calibration done on the same plate. The affinity constant K_D was calculated from the relevant Scatchard plot.

Inhibition Assays:

The inhibition assay was performed in microtiter plates coated with HBs particles (2 µg/ml in PBS). The plate was blocked with 3% BSA in PBS. Hybridoma supernatants containing anti HBs antibodies were serially diluted. 50 µl of each dilution were added to the coated microtiter wells. Subsequently, 50 µl of HBs particles (ad/ay, 0.5 µl/ml in PBS) or PBS alone were added to each well. The plates were incubated overnight at room temperature in a humid chamber and washed 5 times with PBS-Tween. Next, 50 µl of goat anti human IgG conjugated to HRP (diluted 1:5000 in PBS) were added to each well. After a 4 hour incubation at room temperature in a humid chamber the plates were washed 5 times with PBS-Tween, and TMB was added to each well. Results were read using an ELISA reader, in a wavelength of 450 nm.

Immunohistostaining:

HBV positive liver fragment was fixed in 4% neutral buffered formaldehyde for 24 h and then embedded in paraffin using routine procedures. Sections of 4 μm thickness were cut from paraffin blocks and mounted on polylysine-coated slides. After deparaffinization and peroxidase quenching staining was performed using our monoclonal Human anti-HBs Protein A-purified antibodies followed by biotinylated Goat anti-Human IgG (H + L) (Zymed, San Francisco, CA) using Histostain-SPTM kit (Zymed) according to the manufacture's recommendation. Control slides without using the 1st Human anti-HBs antibody were stained in parallel.

Sequence analysis:

Total RNA was isolated from 10 x 10^6 hybridoma cells with RNAsol B reagent (TEL-TEX, Inc. Friendswood, Texas). cDNA was prepared from 10μg of total RNA with reverse transcriptase and oligo dT (Promega, Madison, WI) according to standard procedures. PCR was performed on 1/50 of the RT reaction mixture with V_H, V_λ or V_κ 5' leader primers and 3' primers corresponding to human constant region. The PCR fragments were cloned into pGEM-T vector (Promega). The inserts were sequenced using an ABI 377 sequencing machine. Sequences were analyzed by comparison to Genbank and by alignment to Kabat sequences (Kabat et al. 1991, Sequences of proteins of immunological interest (5^{th} Ed.) U.S. Dept. of Health and Human Services, National Institutes of Health, Bethesda, MD).

Example 1: Production of human anti HBs antibodies in chimeric mice

Human peripheral blood lymphocytes (PBL) from donors positive for anti HBs antibodies were implanted intraperitoneally into irradiated BALB/C mice which were radioprotected by transplantation of bone marrow from SCID mice. These chimeric mice were immunized with Hepatitis B vaccine (Engerix B) to induce a secondary immune response. The production of specific anti HBs antibodies along with total human Ig secretion was measured in mice sera. Fig. 1 shows levels of total human Ig and specific anti HBs antibodies in mice sera 14 days after transplantation of human PBL. Although the levels of human Ig secreted are

similar in immunized and control mice, a strong specific immune response develops in mice vaccinated with hepatitis B vaccine as compared to the control group. Comparison of the levels of specific human antibodies produced in response to the antigen in immunized mice to their levels in the donors sera, indicates a 5-10 fold increase in the mice. Moreover, the specific activity measured in mice sera, i.e. the levels of anti HBs specific antibodies per mg of human Ig secreted, is 102-104 fold higher than the specific activity observed in the donor. This increase demonstrates a very high amplification of anti HBs antibody production in response to the antigen in the chimeric mice (Fig. 2). Production of human antibodies is detectable 10 days after immunization and reaches a plateau after three weeks. The specific activity is high at day 13 after immunization and decreases thereafter (due to increase in total human Ig secretion) (Fig. 3).

Example 2: Preparation and characterization of human monoclonal antibodies against HBs

Human B cells harvested from mice spleens two weeks after immunization were fused to human- mouse heteromyeloma cells (Posner *et al. Supra*). Hybridoma cells were tested for their growth rate, total Ig secretion and specific antibody production. Control fusion experiments were performed on the donor PBL that were activated in vitro with PWM and HBVsAg. Fusion frequencies in different experiments range from 0.9 -5 x 10-5. Most of the growing hybridoma clones secrete human Ig of which 0.1-4 % produce specific human anti HBs antibodies. Anti-HBs secreting hybridoma cells derived from chimeric mice spleens were compared to those obtained from fusion of the donors *in vitro* activated PBL in terms of Ig type and stability as seen in Table 1 below. The majority of the hybridomas from chimeric mice were found to be IgG type and all were stable for more than 12 months. In contrast, hybridomas derived from donor PBL were mostly unstable, only one clone has been stable for more than 12 months. Two stable hybridoma clones that secrete specific human anti HBs monoclonal antibodies were characterized. As seen in Table 2 below, these antibodies were purified on a protein A column as well as on an anti human Ig - agarose column and were both found to be of IgG1 subclass. Affinity constants range from $1.3x10^{-9}$ M to $6x10^{-9}$ M as tested by competitive ELISA. Specificity was tested by competitive inhibition assay using HB surface antigen of the ad-ay (1:1) subtype (Fig. 4). Fig. 5 shows specific binding of the human MoAbs of the invention to HBV by staining human liver fragments infected with HBV.

The gene encoding the variable region of Ab 19.79.5 was isolated, fully sequenced, and its subgroups and CDRs were determined.

The antibody has a fully human Ig gene sequence as determined by alignment to Genebank sequences and Kabat protein sequences. Fig. 10 shows the nucleotide sequence of the cDNA encoding the light chain of the variable region of Ab 19.79.5 and its corresponding amino acid sequence (Sequence identification nos. 1 and 3). Fig. 11 shows the nucleotide sequence of the cDNA encoding the heavy chain of the variable region of Ab 19.79.5 and its corresponding amino acid sequence (Sequence identification nos. 2 and 4).

The sequencing data reveled that the variable region of Ab 19.79.5 consists of the subgroups V_{H3}, J_{H2}, $V_{\lambda 3}$ and $J_{\lambda 3}$.

HBV genomes are classified into six groups A to F, based on the degree of similarity in their nucleotide sequences. The genetic variability of HBV is further reflected in the occurrence of different serotypes of HBsAg. The common determinant '*a*' and two pairs of mutually exclusive determinants '*d/y*' and '*w/r*' enable the distinction of four major subtypes of HBsAg: *adw, adr, ayw* and *ayr*. Additional determinants designated subdeterminants of *w* (*w*1 to *w*4) have allowed the definition of four serotypes of *ayw* (*ayw*1-4) and two serotypes of *adw*, i.e. *adw*2 and *adw*4. Additional subtype variation is added by the *q* determinant, which is present on almost all subtypes. Its absence is marked by a '*q*-' sign.

The kind of HBV serotypes recognized by Ab 19.79.5 was examined using a set of 15 different HBsAg types (Norder et al., 1992, Journal of General Virology, 73, 3141; Magnius and Norder, 1995, Intervirology, 38, 24-34). As can be seen in Fig. 6, Ab 19.79.5 has a complex recognition pattern of the different HBsAg serotypes.

Example 3: Biological activity of human monoclonal antibodies against HBs

The biological activity of Ab 19.79.5 and Ab 18.5.1013 was characterized using the following HBV animal model: a mouse was treated so as to allow the stable engraftment of human liver fragments. The treatment included intensive irradiation followed by transplantation of scid (severe combined immunodeficient) mice bone marrow. Viral infection of human liver fragments was performed ex-vivo using HBV positive human serum (EP 699 235).

The animal model was used in three different modes representing various potential uses of the antibodies: inhibition of infection mode, combined prophylaxis/inhibition mode and combined inhibition/treatment.

1. Inhibition mode - This model demonstrates the ability to use the antibody to inhibit liver infection by HBV. HBV positive human serum was preincubated with Ab 18.5.1013, followed by standard ex-vivo liver infection. HBV-DNA in mice sera was tested 11 and 18 days after transplantation. As seen in Fig. 7 there was a significant reduction in the percentage of infected animals in the antibody treated group as compared to the untreated group.

2. Combined prophylaxis/inhibition mode - This model represents liver transplantation. In this model mice were treated with Ab 19.79.5(10 I.U./mouse) three days before liver transplantation followed by transplantation of human liver fragments which were ex vivo infected with HBV in the presence of Ab 19.79.5 (100 I.U.). HBV DNA was tested in mice sera 10 and 17 days after transplantation. As can be seen in Fig. 8, there was a significant reduction in the percentage of infected animals in the treated group compared to the control group.

3. Combined inhibition/treatment mode - a) HBV positive human serum was preincubated with Ab 19.79.5 followed by standard *ex vivo* liver infection. b) Mice were treated with Ab 19.79.5 at days 0 and 7 post transplantation. HBV DNA in mice sera was tested on days 11 and 19. As can be seen in Fig. 9, the percentage of infected animals in the Ab 19.79.5 treated group was significantly reduced but rebounded about two weeks after the treatment was stopped.

Example 4: Combination therapy of human monoclonal antibodies against HBs and an anti viral agent

Using the HBV model described above, mice are treated with an anti viral drug (a nucleoside analogue, 0.5 mg/mouse/day) at days 17-20 post transplantation. A group of mice is further treated with the human monoclonal antibodies of the invention at days 19 and 20. The presence of HBV DNA in mice sera is tested on days 21 and 27.

Table 1

Stability	Anti-HBs Secretors		Source of Hybridoma Cells
	IgM	IgG	
1 stable for > 10 months 47 unstable	25 (52%)	23 (48%)	*In Vitro* Activated PBL
6 stable for > 10 months 3 unstable	3 (33%)	6 (67%)	Chimeric Mouse Splenocytes

Table 2

Kd (M)	Production $\mu g/10^5$ cells/day	Type	Clone
6.1×10^{-9}	10.3	IgG1 Vλ	18.5.1013
1.62×10^{-9}	5.8	IgG1 Vλ	19.79.5

CLAIMS:

1. A process for obtaining human monoclonal antibodies (hMoAb) capable of binding to Hepatitis B virus surface antigen (HBVsAg) comprising:

(a) immunizing a chimeric rodent M4 having xenogeneic hematopoietic cells with Hepatitis B surface antigen HBVsAg such that xenogeneic antibody-producing cells are produced in said rodent, wherein said rodent M4 is a rodent M1, the hematopoietic cells of which have been substantially destroyed, said rodent M1 having transplanted therein hematopoietic cells derived from a mouse M2 having a hematopoietic deficiency, and xenogeneic hematopoietic cells derived from human M3;

(b) removing and immortalizing said antibody-producing cells;

(c) selecting and cloning the immortalized antibody producing cells producing the antibodies capable of binding to HBVsAg and;

(d) isolating the antibodies produced by the selected, cloned immortalized antibody producing cells.

2. A process according to Claim 1, wherein the rodent M1 is a BALB/C mouse and the mouse M2 is a SCID mouse.

3. A process according to Claim 1 or 2, wherein the human M3 is human having a high level of anti HBVsAg antibody and said xenogeneic hematopoietic cells derived from human M3 are peripheral blood lymphocytes (PBL).

4. A process according to Claims 1-3, wherein the Hepatitis B surface antigen is Engerix™-B vaccine.

5. A human monoclonal antibody being selected from the group consisting of:

(a) the monoclonal antibody 18.5.1013 which is secreted by the hybridoma cell line deposited in the European Collection of Cell Cultures (ECACC) under Accession No. 96052170;

(b) fragments of the antibody of (a) which substantially retain the antigen binding characteristics of the whole antibody.

6. A human monoclonal antibody being selected from the group consisting of:

(a) the monoclonal antibody 19.79.5 which is secreted by the

hybridoma cell line deposited in the European Collection of Cell Cultures (ECACC) under Accession No. 96052168;

(b) fragments of the antibody of (a) which substantially retain the antigen binding characteristics of the whole antibody.

7. The hybridoma cell line deposited at the ECACC on May 22, 1996 under Accession No. 96052170.

8. The hybridoma cell line deposited at the ECACC on May 22, 1996 under Accession No. 96052168.

9. A pharmaceutical composition for the prevention and/or treatment of HBV infections comprising as active ingredient an antibody in accordance with Claim 5 and/or 6 together with a pharmaceutically acceptable carrier.

10. A method for the treatment of HBV infections comprising administering to an individual in need a therapeutically effective amount of antibodies according to Claim 5 and/or 6.

11. A method for the prevention of HBV infections comprising administering to an individual an antibody in accordance with Claim 5 and/or 6 to prevent further infection of the treated individual with HBV.

12. A method for the diagnosis of HBV infections in a body fluid sample comprising:

(a) contacting said sample with an antibody of any of Claim 5 or 6 under conditions enabling the formation of antibody-antigen complexes;

(b) determining the level of antibody-antigen complexes formed;

a level significantly higher than that formed in a control sample indicating an HBV infection in the tested body fluid sample.

13. Use of an antibody in accordance with claim 5 or 6 in combination with an anti viral agent for the prevention and/or treatment of HBV infection.

14. Use of an antibody in accordance with claim 13 wherein said anti viral agent is selected from the group consisting of: interferons, anti HB polyclonal antibodies, nucleoside analogues and inhibitors of DNA polymerase.

15. A pharmaceutical composition for the prevention and/or treatment of HBV infections comprising as an active ingredient at least one antibody in accordance

with claim 5 or 6 in combination with at least one other active ingredient being an anti viral agent.

16. A pharmaceutical composition according to claim 15 wherein the anti viral agent is selected from the group consisting of: interferons, anti HB polyclonal antibodies, nucleoside analogues and inhibitors of DNA polymerase.

17. A method for the prevention and/or treatment of HBV infections comprising administering to an individual in need a therapeutically effective amount of a pharmaceutical composition according to any one of claims 9, 15 or 16.

18. Use of an antibody in accordance with claim 5 or 6 for the manufacture of a pharmaceutical composition for the prevention and/or treatment of HBV infections.

CLAIMS:

1. A process for obtaining human monoclonal antibodies (hMoAb) capable of binding to Hepatitis B virus surface antigen (HBVsAg) comprising:

(a) immunizing a chimeric rodent M4 having xenogeneic hematopoietic cells with Hepatitis B surface antigen HBVsAg such that xenogeneic antibody-producing cells are produced in said rodent, wherein said rodent M4 is a rodent M1, the hematopoietic cells of which have been substantially destroyed, said rodent M1 having transplanted therein hematopoietic cells derived from a mouse M2 having a hematopoietic deficiency, and xenogeneic hematopoietic cells derived from human M3;

(b) removing and immortalizing said antibody-producing cells;

(c) selecting and cloning the immortalized antibody producing cells producing the antibodies capable of binding to HBVsAg and;

(d) isolating the antibodies produced by the selected, cloned immortalized antibody producing cells.

2. A process according to Claim 1, wherein the rodent M1 is a BALB/C mouse and the mouse M2 is a SCID mouse.

3. A process according to Claim 1 or 2, wherein the human M3 is human having a high level of anti HBVsAg antibody and said xenogeneic hematopoietic cells derived from human M3 are peripheral blood lymphocytes (PBL).

4. A process according to Claims 1-3, wherein the Hepatitis B surface antigen is Engerix™-B vaccine.

5. A human monoclonal antibody obtained by the process of Claim 1 and fragments thereof substantially maintaining the antigen binding characteristics of said antibodies.

6. A hybridoma cell line producing a human monoclonal antibody in accordance with Claim 5.

7. A human monoclonal antibody being selected from the group consisting of:

(a) the monoclonal antibody 18.5.1013 which is secreted by the hybridoma cell line deposited in the European Collection of Cell Cultures (ECACC) under Accession No. 96052170;

(b) an antibody capable of binding to the antigen which is bound by said 18.5.1013 antibody; and

(c) fragments of the antibodies of (a) or (b) which substantially

retain the antigen binding characteristics of the whole antibodies.

8. A human monoclonal antibody being selected from the group consisting of:

(a) the monoclonal antibody 19.79.5 which is secreted by the hybridoma cell line deposited in the European Collection of Cell Cultures (ECACC) under Accession No. 96052168;

(b) an antibody capable of binding to the antigen which is bound by said 19.79.5 antibody; and

(c) fragments of the antibodies of (a) or (b) which substantially retain the antigen binding characteristics of the whole antibodies.

9. The hybridoma cell line deposited at the ECACC on May 22, 1996 under Accession No. 96052170.

10. The hybridoma cell line deposited at the ECACC on May 22, 1996 under Accession No. 96052168.

11. An antigen bound by an antibody according to Claim 7 or 8.

12. A pharmaceutical composition for the treatment of HBV infections comprising as an active ingredient an antibody in accordance with Claims 5, 7 or 8 together with a pharmaceutically acceptable carrier.

13. A method for the treatment of HBV infections comprising administering to an individual in need a therapeutically effective amount of antibodies according to Claims 5, 7 and 8.

14. A method for the prevention of HBV infections comprising administeri ng to an individual antibodies in accordance with Claims 5, 7 or 8 to prevent further infection of the treated individual with HBV.

15. A method for the prevention of HBV infections comprising immunizing an individual with an antigen according to Claim 11.

16. A method for the diagnosis of HBV infections in a body fluid sample comprising:

(a) contacting said sample with an antibody of any of Claims 5, 7 or 8 under conditions enabling the formation of antibody-antigen complexes;

(b) determining the level of antibody-antigen complexes formed;

a level significantly higher than that formed in a control sample indicating an HBV infection in the tested body fluid sample.

17. A method for detecting anti HBV antibodies in a body fluid sample comprising:

(a) contacting the sample with the antigen of Claim 11 under

conditions enabling the formation of antibody-antigen complexes;

(b) determining the level of antibody-antigen complexes formed; and

(c) indicating the presence of HBV antigens in the tested body fluid sample.

18. A kit for use in the method of Claim 16.

19. A kit for use in the method of Claim 17.

20. Use of an antibody in accordance with any one of claims 5, 7, or 8 in combination with an anti viral agent for the treatment of HBV infection.

21. Use of an antibody in accordance with claim 20 wherein said viral agent is selected from the group consisting of: interferons, anti HB monoclonal antibodies, anti HB polyclonal antibodies, nucleoside analogues and inhibitors of DNA polymerase.

22. A pharmaceutical composition for the treatment of HBV infections comprising as an active ingredient at least one antibody in accordance with claims 5, 7, or 8 adopted for use in combination with at least one other active ingredient being an anti viral agent.

23. A pharmaceutical composition according to claim 22 wherein the anti viral agent is selected from the group consisting of: interferons, anti HB monoclonal antibodies, anti HB polyclonal antibodies, nucleoside analogues and inhibitors of DNA polymerase.

A method for the treatment of HBV infections comprising administering to an individual in need a therapeutically effective amount of a pharmaceutical composition according to claims 22 or 23. .24

ABSTRACT

Disclosed is a process for obtaining hybridoma cell lines which produce human antibodies capable of binding to the hepatitis B virus surface antigen (HBVsAg), as well as the hybridoma cell lines, and antibodies produced by the cell lines. Also disclosed are various uses of said antibodies in the prevention and treatment of HBV infection. Peripheral blood lymphocytes obtained from human donors having a high titer of anti HBVsAg antibodies are engrafted into normal strains of mice which were lethally irradiated and radioprotected with SCID bone marrow. After immunization of such chimeric mice with HBVsAg, human cells are obtained from the mice spleens and fused in vitro with heteromyeloma cells to generate hybridomas secreting human antibodies having a high affinity and specificity to HBVsAg.

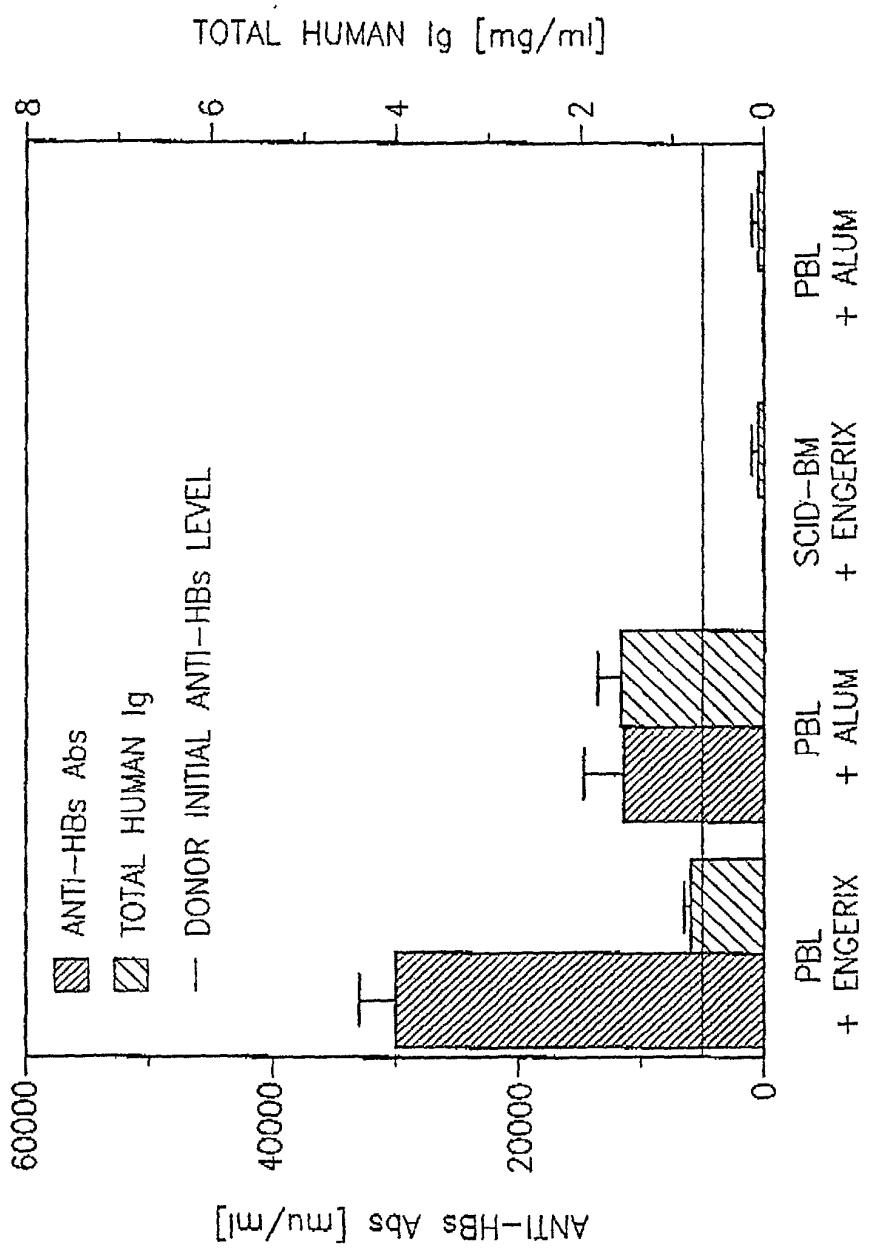

TOTAL HUMAN Ig [mg/ml]
8
6
4
2
0
ANTI-HBs Abs
TOTAL HUMAN Ig
DONOR INITIAL ANTI-HBs LEVEL
60000
40000
20000
0
ANTI-HBs Abs [mu/ml]
PBL + ENGERIX
PBL + ALUM
SCID-BM + ENGERIX
PBL + ALUM

FIG.1

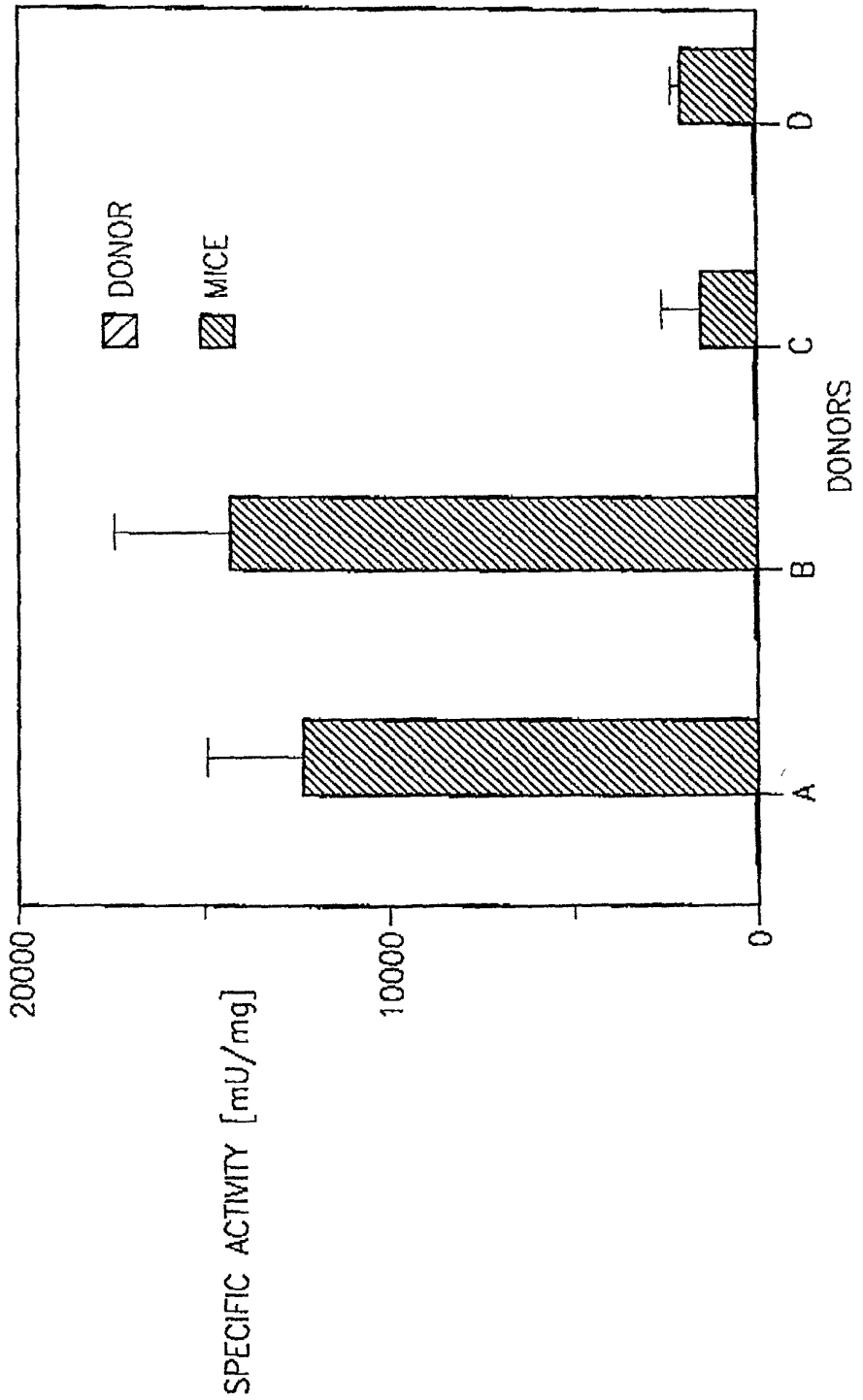

DONOR
MICE
SPECIFIC ACTIVITY [mU/mg]
20000
10000
0
A
B
C
D
DONORS

FIG.2

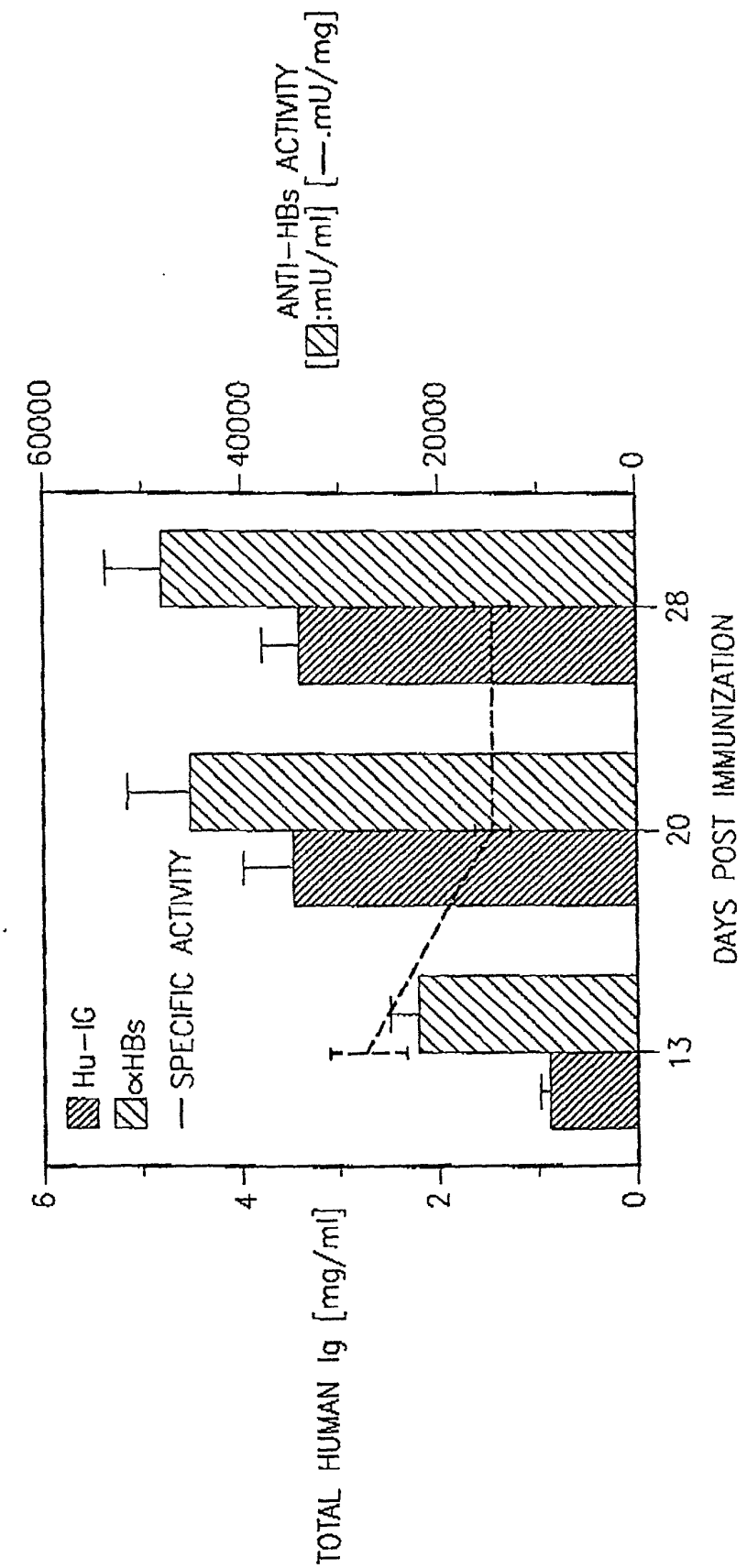

ANTI-HBs ACTIVITY
[▨:mU/ml] [—.mU/mg]
60000
40000
20000
0
28
20
13
DAYS POST IMMUNIZATION
Hu-IG
αHBs
— SPECIFIC ACTIVITY
6
4
2
0
TOTAL HUMAN Ig [mg/ml]

FIG.3

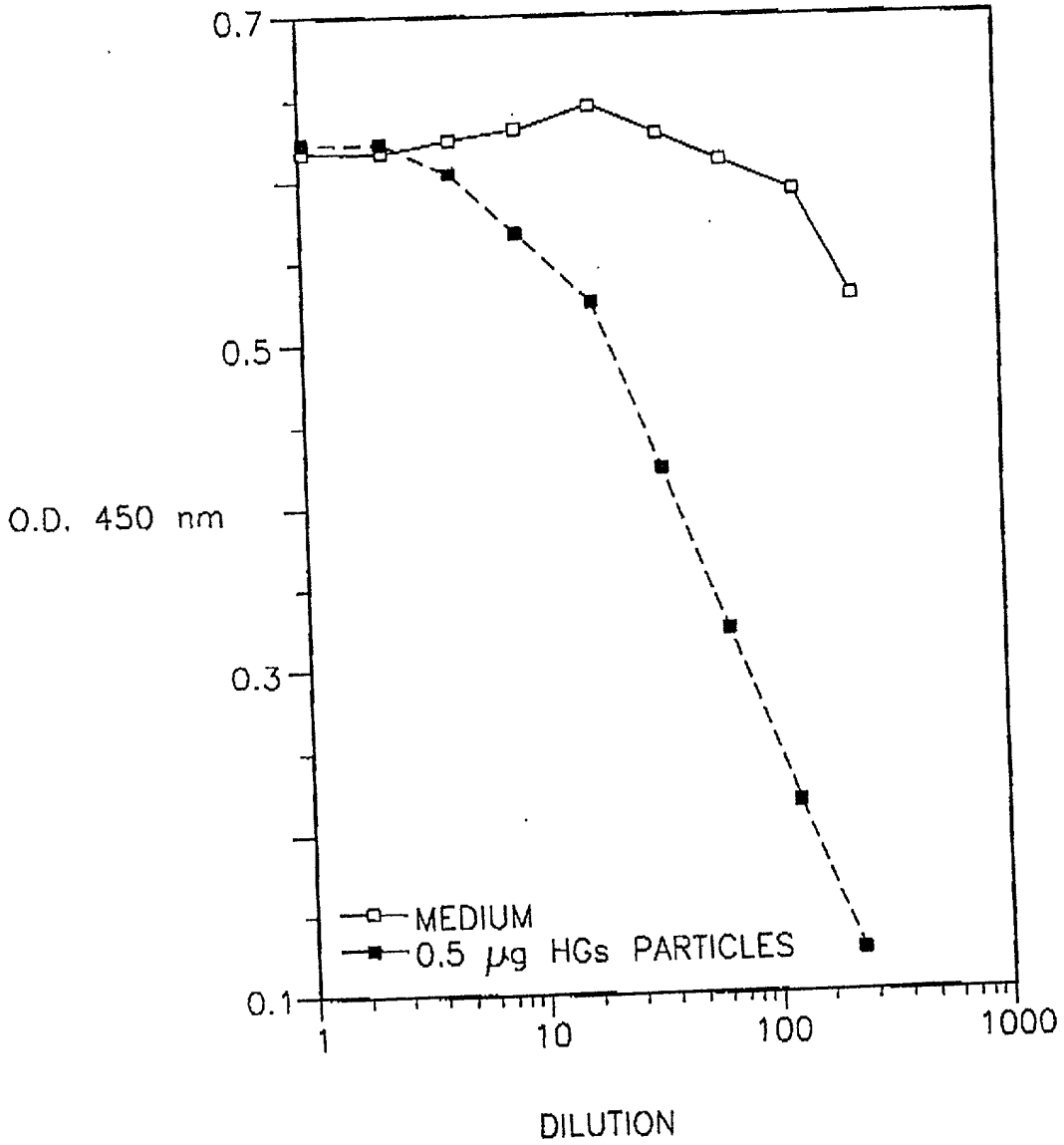

0.7
0.5
0.3
0.1
O.D. 450 nm
MEDIUM
0.5 μg HGs PARTICLES
1
10
100
1000
DILUTION

FIG.4

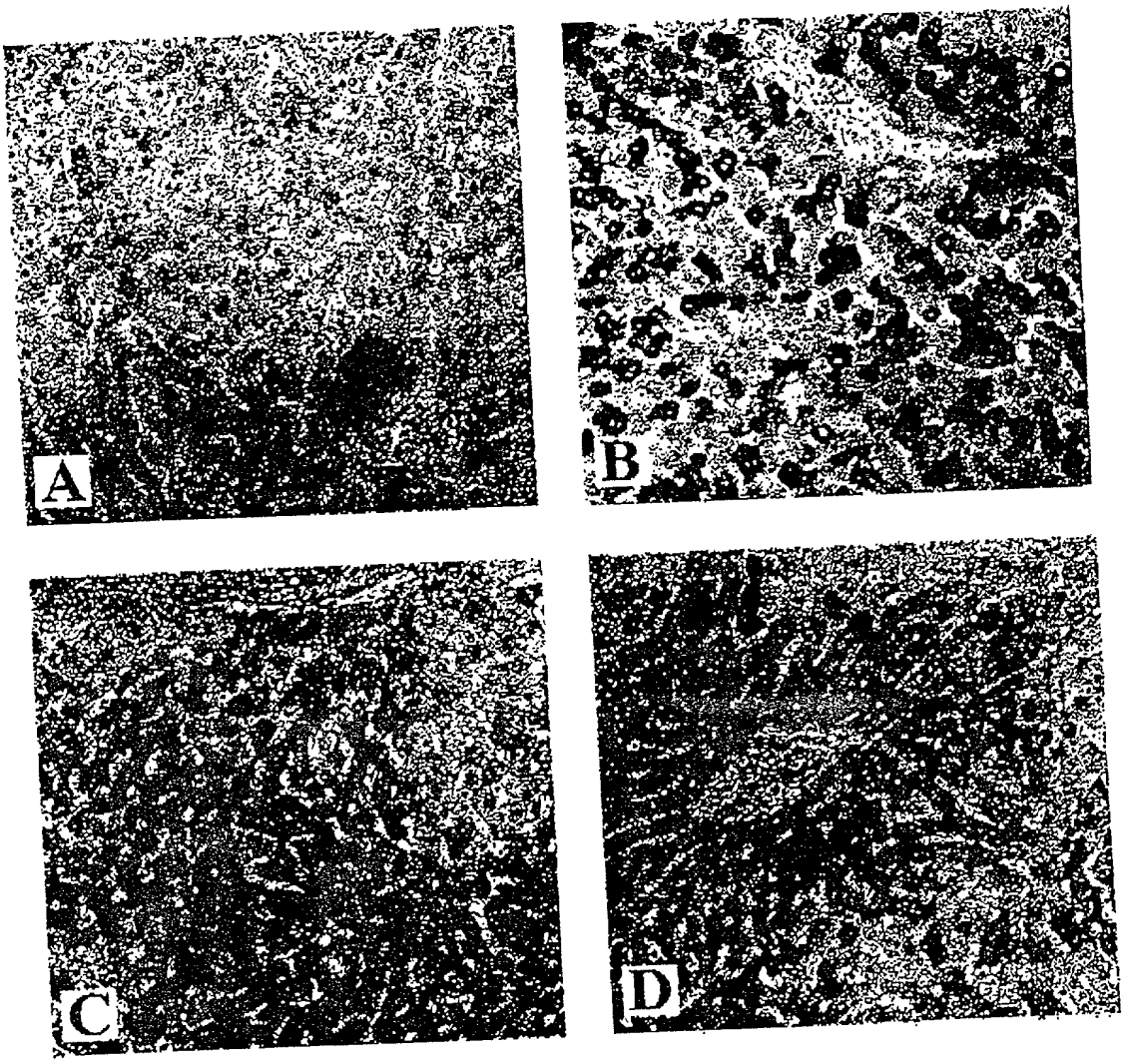
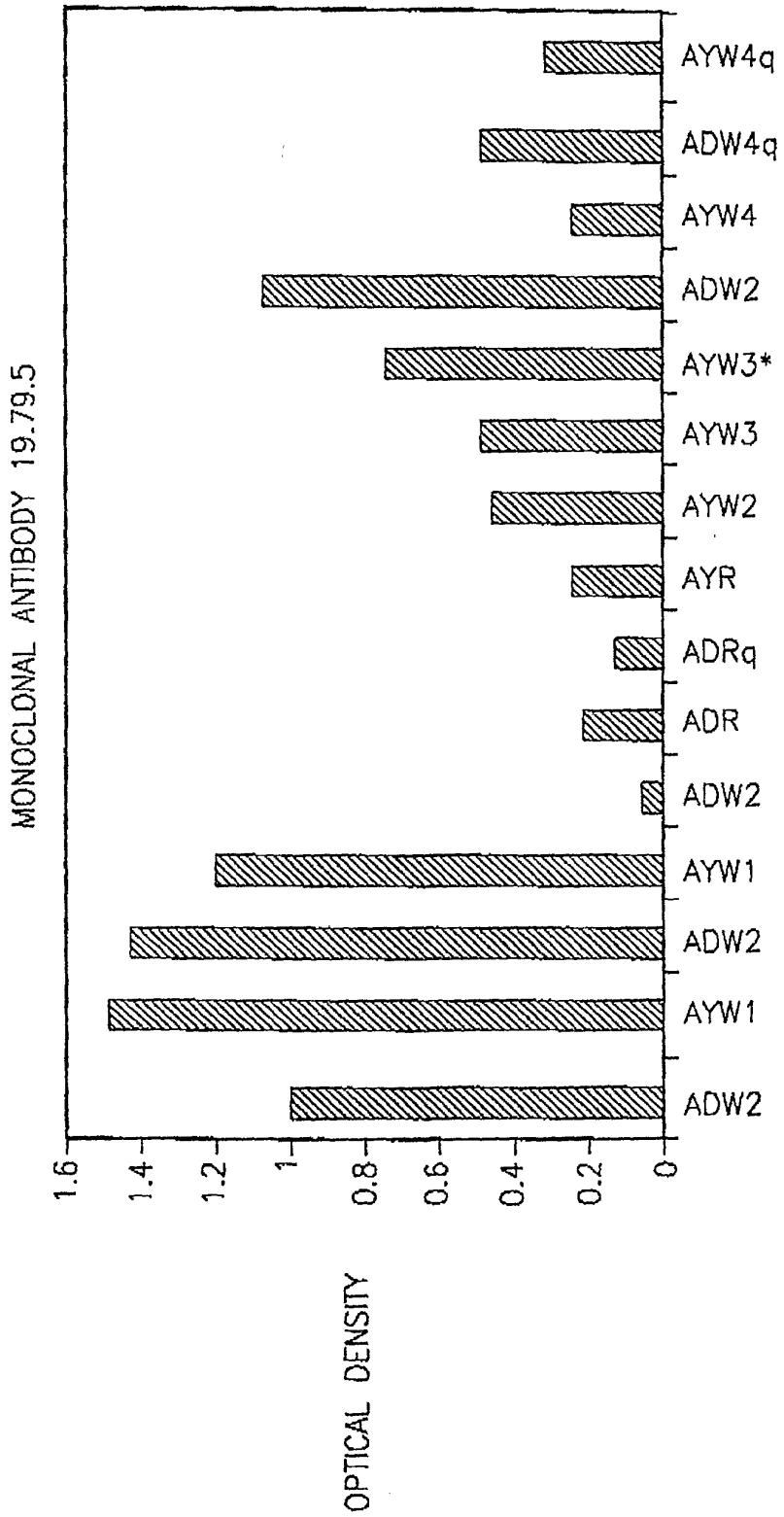


FIG.5


MONOCLONAL ANTIBODY 19.79.5
AYW4q
ADW4q
AYW4
ADW2
AYW3*
AYW3
AYW2
AYR
ADRq
ADR
ADW2
AYW1
ADW2
AYW1
ADW2
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1.4
1.2
1
0.8
0.6
0.4
0.2
0
OPTICAL DENSITY

FIG.6

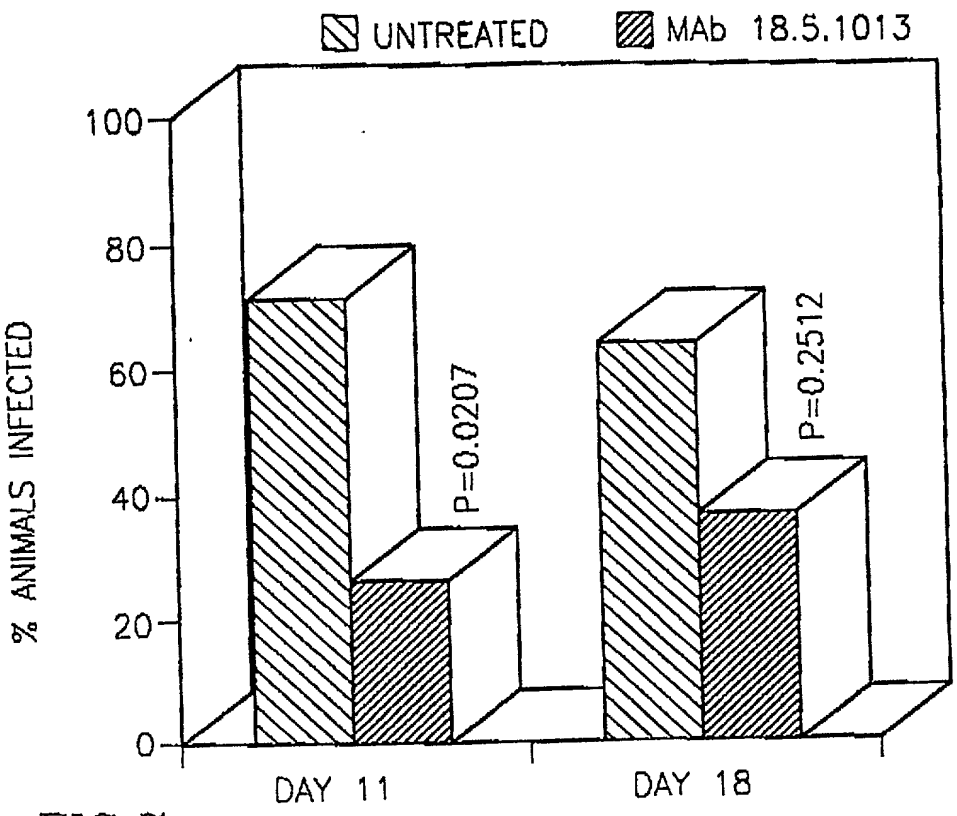
UNTREATED
MAb 18.5.1013
100
80
60
40
20
0
% ANIMALS INFECTED
P=0.0207
P=0.2512
DAY 11
DAY 18

FIG.7

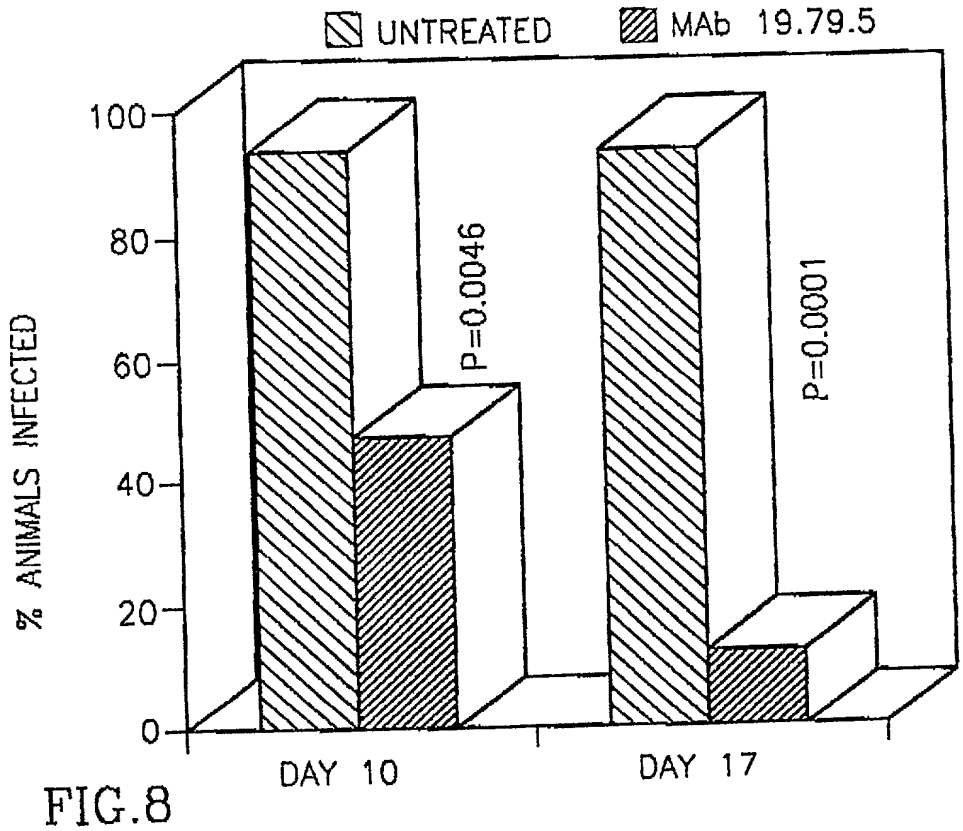
UNTREATED
MAb 19.79.5
100
80
60
40
20
0
% ANIMALS INFECTED
P=0.0046
P=0.0001
DAY 10
DAY 17

FIG.8

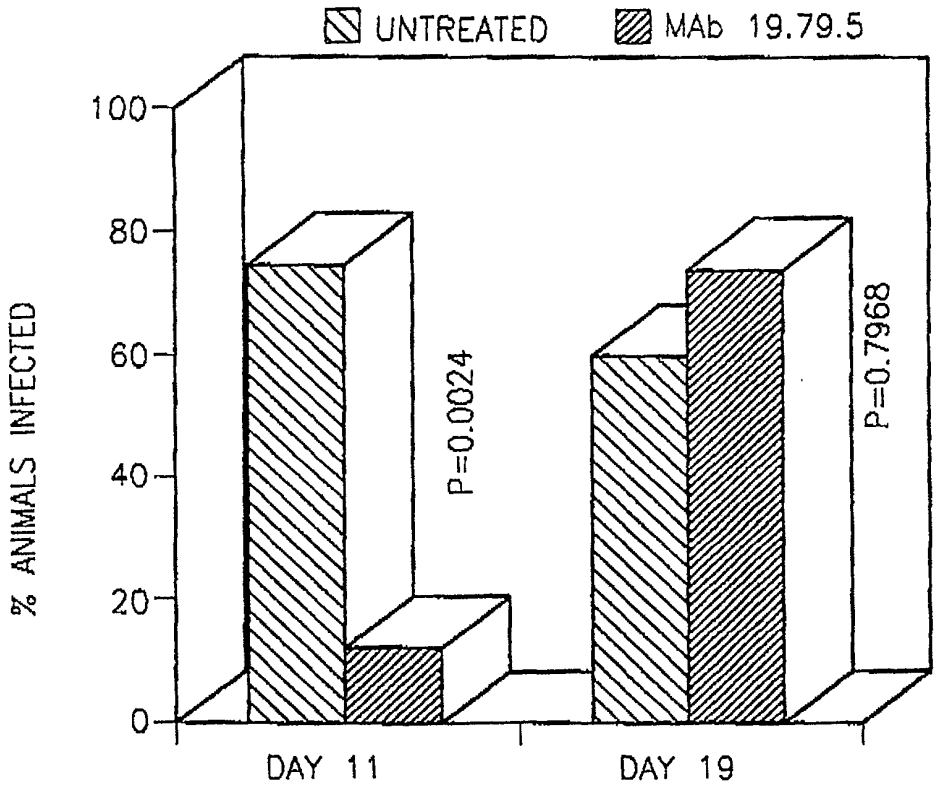
UNTREATED
MAb 19.79.5
% ANIMALS INFECTED
100
80
60
40
20
0
P=0.0024
P=0.7968
DAY 11
DAY 19

FIG.9

		Ser TCC	Tyr TAT	Val GTG 9	Leu CTG	Thr ACT	Gln CAG 18	Pro CCA	Pro CCC	Ser TCG 27	Val GTG	Ser TCA	Val GTG 36	Ala GCC	Pro CCA	Gly GGA 45	Lys AAG	Thr ACG	Ala GCC 54
Arg AGG	Ile ATT	Ser TCC 63	Cys TGT	Gly GGG	Gly GGA 72	Asn AAC	Asn AAC	Ile ATT 81	Gly GGA	Thr ACT	Lys AAA 90	Asn AAT	Val GTG	His CAC 99	Trp TGG	Tyr TAC	Gln CAG 108	Gln CAG	Lys AAG
Pro CCA	Gly GGC	Gln CAG 123	Ala GCC	Pro CCT	Val GTG 132	Leu CTG	Val GTC	Val GTC 141	Tyr TAT	Ala GCT	Asp GAT 150	Ser AGC	Asp GAC	Arg CGG 159	Pro CCC	Ser TCA	Gly GGG 168	Ile ATC	Pro CCT
Glu GAG	Arg CGA	Phe TTC 183	Ser TCT	Gly GGC	Ser TCC 192	Asn AAC	Ser TCT	Gly GGG 201	Asn AAC	Thr ACG	Ala GCC 210	Thr ACC	Leu CTG	Thr ACC 219	Ile ATC	Ser AGC	Arg AGG 228	Val GTC	Glu GAA
Val GTC	Gly GGG	Asp GAT 243	Glu GAG	Ala GCC	Asp GAC 252	Tyr TAT	Tyr TAC	Cys TGT 261	Gln CAG	Val GTG	Trp TGG 270	Asp GAT	Ser AGT	Val GTT 279	Ser AGT	Tyr TAT	His CAT 288	Val GTG	Val GTA
Phe TTT	Gly GGC	Gly GGA 303	Gly GGG	Thr ACC	Thr ACG 312	Leu CTG	Thr ACC	Val GTC 321	Leu CTA	Gly GGT									

FIG.10

		Gln CAG	Val GTG	Gln CAG 9	Leu CTG	Val GTG	Glu GAG 18	Ser TCT	Gly GGG	Gly GGA 27	Gly GGC	Val GTG	Val GTC 36	Gln CAG	Pro CCT	Gly GGG 45	Gly GGG	Ser TCC	Leu CTG 54
Arg AGA	Leu CTC	Ser TCC 63	Cys TGT	Ala GCA	Pro CCG 72	Ser TCT	Gly GGA	Phe TTC 81	Val GTC	Phe TTC	Arg AGG 90	Ser AGT	Tyr TAT	Gly GGC 99	Met ATG	His CAC	Trp TGG 108	Val GTC	Arg CGC
Gln CAG	Thr ACT	Pro CCA 123	Gly GGC	Lys AAG	Gly GGG 132	Leu CTT	Glu GAG	Trp TGG 141	Val GTG	Ser TCA	Leu CTT 150	Ile ATA	Trp TGG	His CAT 159	Asp GAT	Gly GGA	Ser AGT 168	Asn AAT	Arg AGA
Phe TTC	Tyr TAT	Ala GCA 183	Asp GAC	Ser TCC	Val GTG 192	Lys AAG	Gly GGC	Arg CGA 201	Phe TTC	Thr ACC	Ile ATC 210	Ser TCC	Arg AGA	Asp GAC 219	Asn AAT	Ser TCC	Lys AAG 228	Asn AAC	Thr ACA
Leu TTG	Tyr TAT	Leu TTG 243	Gln CAA	Met ATG	Asn AAC 252	Ser AGC	Leu CTG	Arg AGA 261	Ala GCC	Glu GAA	Asp GAC 270	Thr ACG	Ala GCT	Met ATG 279	Tyr TAC	Phe TTC	Cys TGT 288	Ala GCG	Arg AGA
Glu GAG	Arg AGG	Leu CTG 303	Ile ATT	Ala GCA	Ala GCA 312	Pro CCT	Ala GCT	Ala GCC 321	Phe TTT	Asp GAC	Leu CTC 330	Trp TGG	Gly GGC	Gln CAG 339	Gly GGA	Thr ACC	Leu CTG 348	Val GTC	Thr ACC
Val GTC	Ser TCC	Ser TCG 363																	

FIG.11

Page 1 of 2 [X] Original [ ] Supplemental Atty.Docket: REISNER=5

Combined Declaration for Patent Application and Power of Attorney

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name; and that I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled
HUMAN MONOCLONAL ANTIBODIES TO THE HEPATITIS B SURFACE ANTIGEN

the specification of which (check one)

[ ] is attached hereto;
[ ] was filed in the United States under 35 U.S.C. §111 on _____, as USSN _____*; or
[XX] was/will be filed in the U.S. under 35 U.S.C. §371 by entry into the U.S. national stage of an international (PCT) application, PCT/IL97/00184; filed 10 June 1997, entry requested on _____*; national stage application received USSN _____*; §371/§102(e) date _____* (*if known), and was amended on 25 June 1998 (if applicable).

(include dates of amendments under PCT Art. 19 and 34 if PCT)

I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above; and I acknowledge the duty to disclose to the Patent and Trademark Office (PTO) all information known by me to be material to patentability as defined in 37 C.F.R. § 1.56.

I hereby claim foreign priority benefits under 35 U.S.C. §§ 119, 365 of any prior foreign application(s) for patent or inventor's certificate, or prior PCT application(s) designating a country other than the U.S., listed below with the "Yes" box checked and have also identified below any such application having a filing date before that of the application on which priority is claimed:

(Number)	(Country)	(Day Month Year Filed)	[X] YES	[ ] NO
118625	Israel	25 June 1998		
(Number)	(Country)	(Day Month Year Filed)	[ ] YES	[ ] NO

I hereby claim the benefit under 35 U.S.C. § 120 of any prior U.S. non-provisional Application(s) or prior PCT Application(s) designating the U.S. listed below, or under § 119(e) of any prior U.S. provisional applications listed below, and, insofar as the subject matter of each of the claims of this application is not disclosed in such U.S. or PCT application in the manner provided by the first paragraph of 35 U.S.C. §112, I acknowledge the duty to disclose to the PTO all information as defined in 37 C.F.R. §1.56(a) which occurred between the filing date of the prior application and the national filing date of this application:

(Application Serial No.)	(Day Month Year Filed)	(Status: patented, pending, abandoned)
(Application Serial No.)	(Day Month Year Filed)	(Status: patented, pending, abandoned)
(Application Serial No.)	(Day Month Year Filed)	(Status: patented, pending, abandoned)

I hereby appoint the following attorneys, with full power of substitution, association, and revocation, to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith.

SHERIDAN NEIMARK, REG. NO. 20,520 - ROGER L. BROWDY, REG. NO. 25,618 - ANNE M. KORNBAU, REG. NO. 25,884
NORMAN J. LATKER, REG. NO. 19,963 - IVER P. COOPER, REG. NO. 28,005 - ALLEN C. YUN, * REG. NO. 37,971*
NICK S. BROMER, REG. NO. 33,478 -* Patent Agent

ADDRESS ALL CORRESPONDENCE TO	DIRECT ALL TELEPHONE CALLS TO:
BROWDY AND NEIMARK, P.L.L.C. 419 Seventh Street, N.W. Washington, D.C. 20004	BROWDY AND NEIMARK (202) 628-5197

The undersigned hereby authorizes the U.S. Attorneys or Agents named herein to accept and follow instructions from XTL BIOPHARMACEUTICALS LIMITED as to any action to be taken in the U.S. Patent and Trademark Office regarding this application without direct communication between the U.S. Attorney or Agent and the undersigned. In the event of a change of the persons from whom instructions may be taken, the U.S. Attorneys or Agents named herein will be so notified by the undersigned.

Page 2 of 2 **Atty.Docket: REISNER=5**
Title:
U.S. Application filed _____, Serial No. _____
PCT Application filed 119 June 1997, Serial No. PCT/IL97/00184

I hereby further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. §1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

FULL NAME OF FIRST INVENTOR	INVENTOR'S SIGNATURE	DATE
Yair REISNER	Y. Reisner	16/11/98
RESIDENCE		CITIZENSHIP
Old Jaffa, Israel		Israel
POST OFFICE ADDRESS		
Mazal Kashat 4, 68037 Old Jaffa, Israel	ISX	
FULL NAME OF SECOND JOINT INVENTOR	INVENTOR'S SIGNATURE	DATE
Shlomo DAGAN		Nov 16-98
RESIDENCE		CITIZENSHIP
Rehovot, Israel		Israel
POST OFFICE ADDRESS		
Bustenai 12, 76289 Rehovot, Israel	ISX	
FULL NAME OF THIRD JOINT INVENTOR	INVENTOR'S SIGNATURE	DATE
RESIDENCE		CITIZENSHIP
POST OFFICE ADDRESS		
FULL NAME OF FOURTH JOINT INVENTOR	INVENTOR'S SIGNATURE	DATE
RESIDENCE		CITIZENSHIP
POST OFFICE ADDRESS		
FULL NAME OF FIFTH JOINT INVENTOR	INVENTOR'S SIGNATURE	DATE
RESIDENCE		CITIZENSHIP
POST OFFICE ADDRESS		
FULL NAME OF SIXTH JOINT INVENTOR	INVENTOR'S SIGNATURE	DATE
RESIDENCE		CITIZENSHIP
POST OFFICE ADDRESS		
FULL NAME OF SEVENTH JOINT INVENTOR	INVENTOR'S SIGNATURE	DATE
RESIDENCE		CITIZENSHIP
POST OFFICE ADDRESS		

ALL INVENTORS MUST REVIEW APPLICATION AND DECLARATION BEFORE SIGNING. ALL ALTERATIONS MUST BE INITIALED AND DATED BY ALL INVENTORS PRIOR TO EXECUTION. NO ALTERATIONS CAN BE MADE AFTER THE DECLARATION IS SIGNED. ALL PAGES OF DECLARATION MUST BE SEEN BY ALL INVENTORS.

Applicant or Patentee: Yair REISNER et al. Attorney's Docket No.: REISNER=5
Appln. or Patent No.: Filed or Issued:
For: HUMAN MONOCLONAL ANTIBODIES TO THE HEPATITIS B SURFACE ANTIGEN

VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL BUSINESS ENTITY STATUS
(37 CFR 1.9(f) and 1.27(c)) - SMALL BUSINESS CONCERN

I hereby declare that I am
[ ] the owner of the small business concern identified below:
[XX] an official of the small business concern empowered to act on behalf of the concern identified below:

NAME OF SMALL BUSINESS CONCERN YEDA RESEARCH AND DEVELOPMENT CO. LTD.
ADDRESS OF SMALL BUSINESS CONCERN P.O. Box 95, Rehovot 76100, Israel

I hereby declare that the above identified small business concern qualifies as a small business concern as defined in 13 CFR 121.12, and reproduced in 37 CFR 1.9(d), for purposes of paying reduced fees to the United States Patent and Trademark Office, in that the number of employees of the concern, including those of its affiliates, does not exceed 500 persons. For purposes of this statement, (1) the number of employees of the business concern is the average over the previous fiscal year of the concern of the persons employed on a full-time, part-time or temporary basis during each of the pay periods of the fiscal year, and (2) concerns are affiliates of each other when either, directly or indirectly, one concern controls or has the power to control the other, or a third party or parties controls or has the power to control both.

I hereby declare that rights under contract or law have been conveyed to and remain with the small business concern identified above with regard to the invention entitled HUMAN MONOCLONAL ANTIBODIES TO THE HEPATITIS B SURFACE ANTIGEN by inventors Yair REISNER and Shlomo DAGAN described in:

[ ] the specification filed herewith with title listed as above.
[XX] application no. _____, filed _____.
[ ] patent no. _____, issued _____.

If the rights held by the above-identified small business concern are not exclusive, each individual, concern or organization having rights in the invention is listed below* and no rights to the invention are held by any person, other than the inventor, who would not qualify as an independent inventor under 37 CFR 1.9(c) if that person made the invention, or by any concern which would not qualify as a small business concern under 37 CFR 1.9(d), or a nonprofit organization under 37 CFR 1.9(e).

*Note: Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities (37 CFR 1.27).

NAME XTL BIOPHARMACEUTICALS LIMITED
ADDRESS Kiryat Weizmann, P.O. Box 370, Rehovot 76100, Israel
[ ] INDIVIDUAL [XX] SMALL BUSINESS CONCERN [ ] NONPROFIT ORGANIZATION
NAME
ADDRESS
[ ] INDIVIDUAL [ ] SMALL BUSINESS CONCERN [ ] NONPROFIT ORGANIZATION

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b))

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING PAULINA BEN-AMI
TITLE OF PERSON SIGNING OTHER THAN OWNER VICE PRESIDENT
ADDRESS OF PERSON SIGNING Yeda Research and Development Co. Ltd. P.O.B 95 Rehovot 76100 I
SIGNATURE Paulina Ben-Ami DATE December 10, 1998

Applicant or Patentee: Yair REISNER et al. Attorney's Docket No.: REISNER-5
Appln. or Patent No.: _____ Filed or Issued: _____
For: HUMAN MONOCLONAL ANTIBODIES TO THE HEPATITIS B SURFACE ANTIGEN

VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL BUSINESS ENTITY STATUS
(37 CFR 1.9(f) and 1.27(c)) - SMALL BUSINESS CONCERN

I hereby declare that I am
[ ] the owner of the small business concern identified below:
[XX] an official of the small business concern empowered to act on behalf of the concern identified below:

NAME OF SMALL BUSINESS CONCERN XTL BIOPHARMACEUTICALS LIMITED
ADDRESS OF SMALL BUSINESS CONCERN Kiryat Weizmann, P.O. Box 370, Rehovot 76100, Israel

I hereby declare that the above identified small business concern qualifies as a small business concern as defined in 13 CFR 121.12, and reproduced in 37 CFR 1.9(d), for purposes of paying reduced fees to the United States Patent and Trademark Office, in that the number of employees of the concern, including those of its affiliates, does not exceed 500 persons. For purposes of this statement, (1) the number of employees of the business concern is the average over the previous fiscal year of the concern of the persons employed on a full-time, part-time or temporary basis during each of the pay periods of the fiscal year, and (2) concerns are affiliates of each other when either, directly or indirectly, one concern controls or has the power to control the other, or a third party or parties controls or has the power to control both.

I hereby declare that rights under contract or law have been conveyed to and remain with the small business concern identified above with regard to the invention entitled HUMAN MONOCLONAL ANTIBODIES TO THE HEPATITIS B SURFACE ANTIGEN by inventors Yair REISNER and Shlomo PAGAN described in:

[ ] the specification filed herewith with title listed as above.
[XX] application no. _____, filed _____.
[ ] patent no. _____, issued _____.

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*Note: Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities (37 CFR 1.27).

NAME YEDA RESEARCH AND DEVELOPMENT CO. LTD.
ADDRESS P.O. Box 95, Rehovot 76100, Israel
[ ] INDIVIDUAL [XX] SMALL BUSINESS CONCERN [ ] NONPROFIT ORGANIZATION
NAME _____
ADDRESS _____
[ ] INDIVIDUAL [ ] SMALL BUSINESS CONCERN [ ] NONPROFIT ORGANIZATION

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b))

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING Mirit Lotan
TITLE OF PERSON SIGNING OTHER THAN OWNER PATENT ATTORNEY
ADDRESS OF PERSON SIGNING XTL BIOPHARMACEUTICALS LIMITED, Kiryat Weizmann, P.O.B.370 Rehovot 76100 Israel
SIGNATURE Mirit Lotan DATE December 10, 1998

TOTAL P.03